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TITLE: Novel Interventions for Heat/Exercise Induced Sudden Death and Fatigue

PRINCIPAL INVESTIGATOR: Susan L. Hamilton, Ph.D. and John Capacchione, M.D.

CONTRACTING ORGANIZATION: Baylor College of Medicine, Houston, TX 77030

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Baylor College of Medicine
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13. SUPPLEMENTARY NOTES

14. ABSTRACT
Our goal is to identify ryanodine receptor type 1 (RYR1), calcium channel, voltage-dependent, L type, alpha 1S subunit (CACNA1S), and calsequestrin 1 (CASQ1) gene mutations associated with enhanced susceptibility to Exertional Heat Stroke (EHS), Exertional Rhabdomyolysis (ER), and Malignant Hyperthermia (MH) by enrolling subjects diagnosed with these conditions and performing genetic screening. During the project period, 8 additional subjects were enrolled, bringing the total to 45: 32 index cases and 13 family members from 3 of the index cases. Of the 32 index cases, 7 subjects have known MH-causative RYR1 gene mutations (Arg163Cys, Gly2434Arg, Arg2454Cys, Arg2163His, and Ala2350Thr), 2 have known MH-associated RYR1 variants, 3 have novel RYR1 variants, 14 have no RYR1 mutations or variants, and 2 are still in progress. One subject had a known MH-causative mutation and a novel variant. Of the 13 family members screened from 3 index cases, 7 family members were found to share the same known MH-causative RYR1 gene mutation. Of the known MH-causative mutations, the Arg2454Cys was identified in an African American with a positive CHCT and a history of ER. Identification of this mutation in a subject with ER strengthens the link between MHS and ER.

Although we have not yet found an AICAR derivative that prevents the hypermetabolic response in mice, we have recently explored the use of other drugs that can reduce RyR1 leak. One such drug is rapamycin. We have shown that rapamycin reduces the probability of a hypermetabolic response in YS mice.

15. SUBJECT TERMS: Exertional Heat Stroke, Exertional Rhabdomyolysis, Malignant Hyperthermia , genetic analysis of type 1 Ryanodine Receptor (RYR1) and 5-Aminoimidazole-4-carboxamide ribotide (AICAR).

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON** USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
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Introduction:

Exertional and/or environmental heat stroke (EHS) and exertional rhabdomyolysis (ER) have been reported in subjects with diagnosis of Malignant Hyperthermia (MH). MH is a life-threatening pharmacogenetic disorder caused by mutations in the ryanodine receptor type 1 gene (RYR1) encoding skeletal muscle calcium release channel. MH has also been associated with the genes encoding the calcium-dependent, L type, alpha 1S subunit (CACNA1S) and calsequestin 1 (CASQ1). Our goal is to identify mutations in these genes associated with enhanced susceptibility to EHS/ER/MH by enrolling subjects diagnosed with these conditions and performing genetic screening.

Body:

Human projects:

To date, 45 subjects diagnosed with MHS, ER and/or EHS and their family members have been enrolled in this study (Table 1). Of these, 32 are index cases and 13 are first degree relatives of 3 of the index cases, BU-02, BU-05 and BU-12 (Figs. 1 and 2). Enrollment of family members is important for studying the genotype and phenotype relationships, and to understand the pathogenic significance of familial variants. During this project period, screening of the RYR1, CACNA1S and CASQ1 genes were continued.

RYR1 gene variants were found in several ER subjects (Table 1). Analysis of various databases, including a database of RYR1 mutations associated with different muscle disorders,[2,3] showed that o**f the 32 index cases, 7 subjects have known MH-causative RYR1 gene mutations (Arg163Cys, Gly2434Arg, Arg2454Cys, Arg2163His, and Ala2350Thr), 2 have known MH-associated RYR1 variants, 3 have novel RYR1 variants, 5 have previously published variants of unknown significance and 14 have no RYR1 mutations or variants. Distribution of these variants can be seen in Figure 3. Of the five common mutations, the Arg2454Cys was identified in an African American subject with a history of ER and a positive caffeine-halothane contracture test (CHCT; the validated diagnostic test for MH susceptibility). The finding of a mutation characterized as causative for MH susceptibility (Arg2454Cys) in a subject with known ER, further strengthens the link between the two disorders.**

One subject, who died of an awake MH-like episode, but was previously known to be MH susceptible, had a Phe41Ser RYR1variant and a Gly2434Arg known MH-causative mutation. Of these two variants, the Gly2434Arg is one of the most common pathogenic mutations in the RYR1 gene. The presence of the second variant may have contributed to the fatal outcome in this case; however the functional significance of the second variant (Phe41Ser) requires further study.

CACNA1S gene variants were found in 7 subjects. All identified CACNA1S variants, except the Val1449Gly, were previously reported in NCBI single nucleotide polymorphisms (SNP) database[1] and in genome database that cover genome of several thousands of healthy individuals.[2] Contribution of the newly identified Val1449Gly variant to ER phenotype is unknown.

Two variants were identified in the CASQ1 gene. The contribution of these variants to the MH/EHS phenotypes is unknown.

Screening is ongoing in two subjects.

Mouse Project: We have continued to screen AICAR derivatives to find a drug that is more potent for prevention of the hypermetabolic response in YS mice. None of the derivatives examined so far block the response of the YS mice to hear. We have, therefore begun to examine the effects of other RyR1 modulators. We have found that rapamycin decreases the probability of an MH-like response to heat. A non-immunosuppressive drug (SLF) which acts on RyR1 in a similar fashion to rapamycin also reduces the probability of an MH response. We are currently working to define the mechanism of action of these drugs.

In a separate line of study we have shown that RyR1 mutations increase susceptibility to statin induced myopathies and malignant neuroleptic syndrome.

Key Research Accomplishments:

Over the course of the project, 45 subjects with a history of EHS/ER or MH were enrolled in this study. The RYR1, CACNA1S and CASQ1 genes were screened in these individuals.

RYR1 gene variants were found in several ER subjects (Table 1). Importantly, over the course of this project, three new variants were identified (Ala2533Thr, Glu4410Asp, and Tyr4850Stop). Additionally, 7 index cases were found to have known MH-causative RYR1 mutations **(Arg163Cys, Gly2434Arg, Arg2454Cys, Arg2163His, and Ala2350Thr)**, and 2 were found to have known MH-associated variants.

The Arg2454Cys, a mutation in the RYR1 known to be causative for MH, was identified in an African American subject with a history of ER and a positive caffeine-halothane contracture test (CHCT; the validated diagnostic test for MH susceptibility). The finding of a mutation

characterized as causative for MH susceptibility in a subject with known ER, further strengthens the link between the two disorders.

One of the subjects with a novel variant (Ala2533Thr) was diagnosed as MH susceptible (MHS) by contracture tests. Another subject, with a different novel variant, (Glu4410Asp) was diagnosed as MH normal by contracture tests. Although highly sensitive, the contracture tests have only 78% specificity.[3]

Given this diagnostic limitation of the contracture test, it is possible that subject carrying the Glu4410Asp variant may actually be at risk for MH, despite his MH normal contracture result. On the other hand, the Glu4410Asp might also be a rare, benign, variant that is not associated with ER and/or MH. Further studies to characterize functional consequences of these mutations and to analyze phenotype-genotype relationship are important to determine whether the newly identified variants contribute to subjects' clinical presentation of ER and or MHS.

Another RYR1 variant, Gly4820Arg, was found in the father of a child who had the same variant and died of an awake MH-like episode. This father was subsequently diagnosed MH susceptible by positive CHCT, and was further diagnosed with central core disease based on his muscle histopathology. This case has since been published.[4] Interestingly, another mutation at the same position, Gly4820Trp, was reported in association with MH susceptibility.[5]

CACNA1S gene variants were found in 7 subjects. Two variants were identified in the CASQ1 gene. The contribution of these variants to the MH/EHS phenotypes is unknown.

In our animal studies we have now identified AICAR, rapamycin and SLF as potential new drugs for prevention of heat induced rhabdomyolysis. We have also shown an association of RyR1 mutations with statin induced myopathies.

Reportable Outcomes:

- Publication: Lavezzi WA, Capacchione JF, Muldoon SM, Sambuughin N, Bina S, Steele D, Brandom BW. Case Report: Death in the Emergency Department: An Unrecognized Awake Malignant Hyperthermia-like Reaction in a Six-Year Old. Anesth Analg. 2013; 116:420-423.
- 45 subjects diagnosed with MHS, ER and/or EHS and their family members have been enrolled (Table 1). Of these, 32 are index cases and 13 are first degree relatives of 3 of the index cases.

- RYR1 gene mutations and variants have been found in 17 index cases which account for 53% of the index cases.
- CACNA1S mutations and variants have been found in 7 index cases.
- CASQ1 mutations and variants have been found in 2 index cases.

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5. Robinson R, Carpenter D, Shaw M-A, Halsall J, Hopkins P (2006) Mutations in *RYR1* in malignant hyperthermia and central core disease. *Hum Mutat* 27:977-89.
6. Sambuughin, N., Liu, X., Bijarnia, S., Wallace, T, Verma, I., Hamilton, S.L., Muldoon, S., Tallon, L. and Wang, S. Exome sequencing reveals SCO2 mutations in a family presented with fatal infantile hyperthermia, Journal of Human Genetics , (31 January 2013) | doi:10.1038/jhg.2012.156. PMID: 23364397 [PubMed - in process]
7. Mark Knoblauch, Adan Dagnino-Acosta, Susan L Hamilton Mice with RyR1 mutation (Y524S) undergo hypermetabolic response to simvastatin. Skeletal Muscle 2013, 3:22 (3 September 2013). http://www.skeletalmusclejournal.com/content/3/1/22

Appendices:

Table 1. Genetic Screening Results

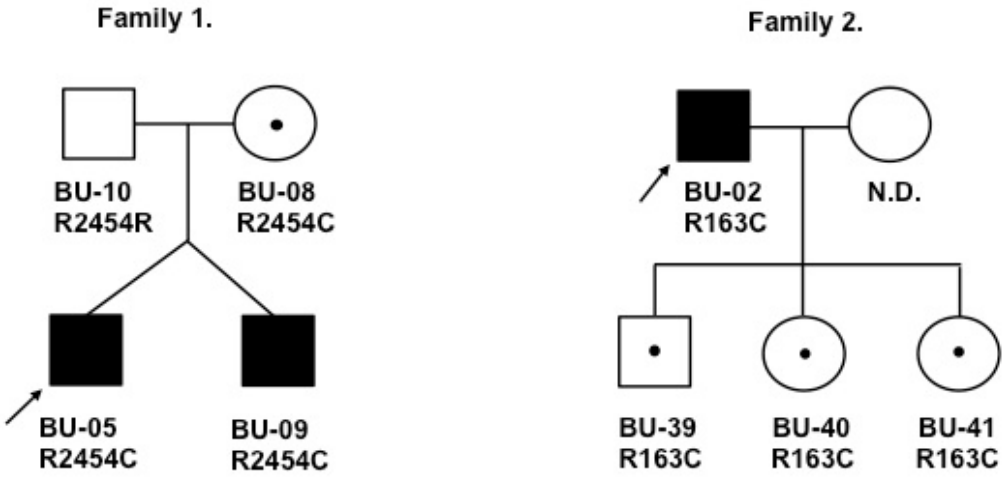
Report ID	Clinical history	CHCT Results	Index or Family members	Results of genetic screening*		
				RYR1	**CACNA1S**	**CASQ1**
BU-01	Exercise induced rhabdomyolysis, heat related death in a family member	Positive	Index	Gly4820Arg	N.A.	N.A.
BU-02	Muscle pain with exercise post MH event	Positive	Index	**Arg163Cys**	N.A.	N.A.
BU-03	Exercise induced rhabdomyolysis, MHS	Positive	Index	Negative	Negative	Negative
BU-04	Exercise and heat intolerance, muscle cramping and MH	Positive	Index	**Asp3986Glu**	N.A.	N.A.
BU-05	Repeated Exercise induced rhabdomyolysis	Positive	Index	**Arg2454Cys**	N.A.	N.A.
BU-06	Repeated Exercise induced rhabdomyolysis	N.D.	Index	**Gly2434Arg**	N.A.	N.A.
BU-07	Death due to MH-like event	N.D.	Index	Negative	N.A.	N.A.
BU-08	No known clinical history	Positive	Mother of BU-05	**Arg2454Cys**	N.A.	N.A.
BU-09	Cardiac arrest during surgery	N.D.	Twin brother of BU-05	**Arg2454Cys**	N.A.	N.A.
BU-10	No known clinical history	N.D.	Father of BU-05	Negative for Arg2454Cys familial mutation	N.A.	N.A.
BU-11	Traumatic MH Episode	N.D.	Index	**Arg2163His**	N.A.	N.A.
BU-12	Death due to MH and Exercise/heat intolerance	N.D.	Index	Phe41Ser **& Gly2434Arg**	N.A.	N.A.
BU-13	MH and heat related death in family member	N.D.	Mother of BU-12	**Gly2434Arg**	N.A.	N.A.
BU-14	Exercise and heat intolerance	Negative	Index	Tyr4850Stop	Arg1658His	Negative
BU-15	Repeated Exertional rhabdomyolysis	Positive	Index	Val4842Met	N.A.	N.A.

BU-16	Exercise and heat intolerance	Negative	Index	Negative	Leu458His Val1449Gly	Negative
BU-17	Repeated Exertional rhabdomyolysis	N.D.	Index	Negative	Negative	Negative
BU-18	Exercise intolerance, heat stroke and mother had suspected MH episode	N.D.	Index	Negative	Negative	Negative
BU-19	MH and heat related death in family member	N.D..	1st Cousin of BU-12	Negative for Gly2434Arg familial mutation	N.A.	N.A.
BU-20	MH and heat related death in family member	N.D.	1st cousin of BU-12	Negative for Gly2434Arg familial mutation	N.A.	N.A
BU-21	Repeated Exertional rhabdomyolysis	Positive	Index	Negative	In progress	
BU-22	MH and heat related death in family member	N.D.	Uncle of BU-12	**Gly2434Arg**	N.A.	N.A.
BU-23	MH and heat related death in family member	N.D.	1st Cousin of BU-12	Negative for Gly2434Arg familial mutation	N.A.	N.A.
BU-24	MH and heat related death in family member	N.D.	1st Cousin of BU-12	Negative for Gly2434Arg familial mutation	N.A.	N.A.
BU-25	Heat and Exercise intolerance	N.D.	Index	Negative	Negative	Negative
BU-26	Exertional Rhabdomyolysis/ Heat & Exercise Intolerance	Negative	Index	In progress		
BU-27	MH and heat related death in family member	N.D.	Twin sister to BU-12	Negative for Gly2434Arg familial mutation	N.A.	N.A.
BU-28	Repeated Exertional rhabdomyolysis	Positive	Index	Negative	Leu1800Ser	Negative
BU-29	Repeated Exertional rhabdomyolysis	Positive	Index	Ala933Thr Ser1342Gly <u>Ala2533Thr</u>	Negative	Negative
BU-30	Repeated Exertional rhabdomyolysis	Positive	Index	Negative	Leu458His	Met87Thr
BU-31	Repeated Exertional rhabdomyolysis	Positive	Index	Negative	Negative	Negative
BU-32	Repeated Exertional rhabdomyolysis	Negative	Index	Arg1109Lys Thr2787Ser	Negative	Negative

BU-33	Repeated Exertional rhabdomyolysis	Negative	Index	Negative	Negative	Negative
BU-34	Repeated Exertional rhabdomyolysis	Negative	Index	Thr4823Met	Arg1658His	Negative
BU-35	Repeated Exertional rhabdomyolysis	Negative	Index	Negative	Arg1539Cys	Negative
BU-36	Repeated Exertional rhabdomyolysis	Negative	Index	Glu4410Asp	Arg1658His	Negative
BU-37	Repeated Exertional rhabdomyolysis	Negative	Index	Met923Val & Pro4501Leu	Negative	Negative
BU-38	MHS, exercise intolerance	Positive	Index	**Gly2434Arg**	N.A.	N.A.
BU-39	Muscle pain with exercise post-MH event; MHS family member	N.D.	Child of BU-02	**Arg163Cys**	N.A.	N.A.
BU-40	Muscle pain with exercise post-MH event; MHS family member	N.D.	Child of BU-02	**Arg163Cys**	N.A.	N.A.
BU-41	Muscle pain with exercise post-MH event; MHS family member	N.D.	Child of BU-02	**Arg163Cys**	N.A.	N.A.
BU-42	Suspicious MH-like clinical episode, heat intolerance, cramping	N.D.	Index	Gln3756Glu	Negative	Tyr140Phe
BU-43	Suspicious MH-like clinical episode	Positive	Index	**Ala2350Thr**	N.A.	N.A.
BU-44	Repeated Exertional rhabdomyolysis	Negative	Index	Negative	Negative	Negative
BU-45	Repeated Exertional rhabdomyolysis	Negative	Index	Negative	Negative	Negative

* - MHS causative mutations are in bold; newly identified amino-acid variants are underlined. N.D. – not determined. N.A. – not applicable

Pedigree Analysis (Figure 1)

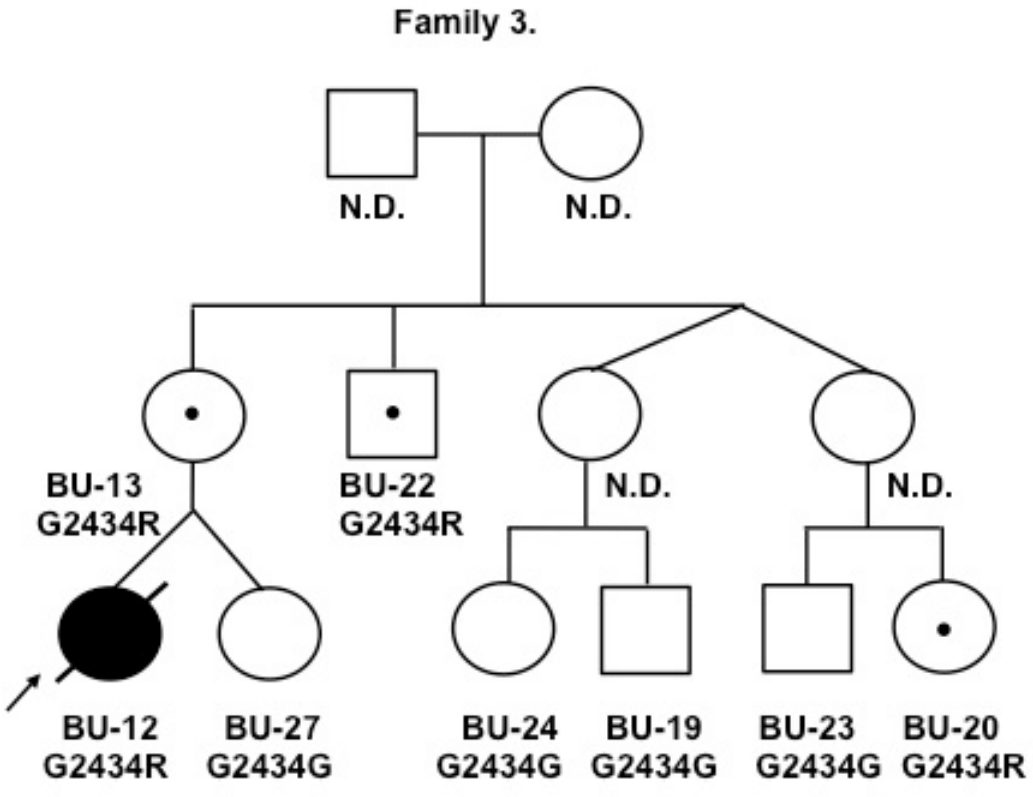


Pedigrees of two unrelated MHS and Exertional Rhabdomyolysis families.

Fully filled symbols denote index cases, while central dots indicate family members that carry the familial mutation. Unfilled indicates that the family member either did not have the mutation, or was not screened in this study.

The “R” and “C” labels denote Arginine and Cysteine respectively. In the cases in this figure, the amino acid changes are at the sequence positions 2454 and 163. “N.D.” indicates that the individual was not screened in this study.

Pedigree Analysis (Figure 2)



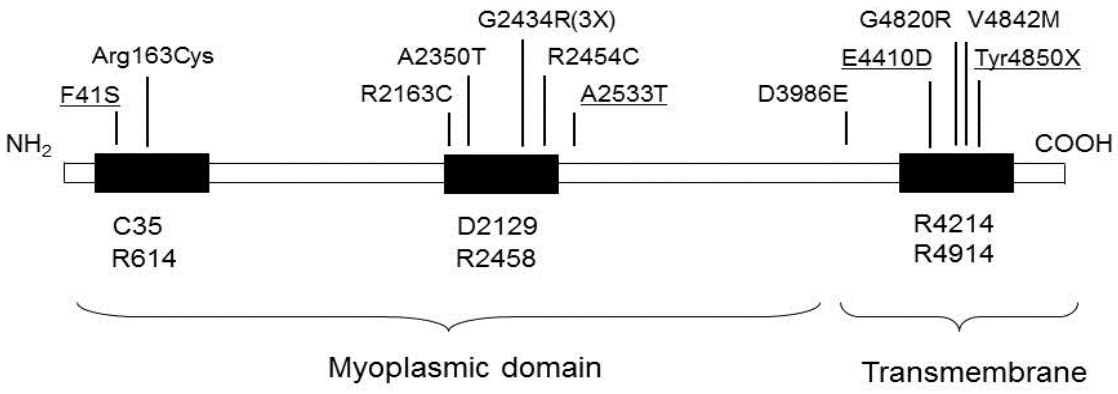
Pedigree of MHS family.

Fully filled symbols denote index cases, while central dots indicate family members that carry the familial mutation. Unfilled symbols indicate that the family member either did not have the mutation, or was not screened in this study.

Filled symbol with bisecting line indicates and individual who died from and MH/ER/EHS event (BU-12).

The “G” and “R” labels denote Glycine and Arginine respectively. In the cases in this figure, the amino acid changes are at the sequence position 2434. “N.D.” indicates that the individual was not screened in this study.

Identified Mutation Distribution (Figure 3)



Distribution of mutations found in the RYR1 gene.

Of the 32 index cases, 7 subjects have known MH-causative RYR1 gene mutations (Arg163Cys, Gly2434Arg, Arg2454Cys, Arg2163His, and Ala2350Thr), 2 have known MH-associated RYR1 variants, 3 have novel RYR1 variants, 5 have previously published variants of unknown significance and 14 have no RYR1 mutations or variants.

QUARTERLY REPORT FORMAT

1. Award No. W81XWH-10-2-0117 **2. Report Date** 10/28/2013

3. Reporting period 7/1/2013 to 9/30/2013

4. PI: Susan Hamilton & John Capacchione **5. Telephone No**. 713-798-5704

6. Institution: Baylor College of Medicine & Uniformed Services University of the Health Sciences (USUHS)

7. Project Title: Novel Interventions for Heat/Exercise Induced Sudden Death and Fatigue

8. Current staff, with percent effort of each on project.

Baylor

Susan Hamilton, Ph.D. 10 %
Dimitra Georgiou, Ph.D. 100 %
Keke Dong 40 %

USUHS

Sheila Muldoon, M.D. 5%
John Capacchione, M.D. 5%
Kelly Dickson 50%
Nyamkhishig Sambuughin, Ph.D. 5%
Francis O'Connor, M.D. 5%
Maria Voelkel 20%

9. Award expenditures to date (as applicable):

	This Qtr/Cumulative		**This Qtr/Cumulative**
Personnel	39,684.81 / 417,511.04	Travel	0 / 5,625.37
Fringe Benefits	9,385.55 / 98,833.61	Equipment	_____/_____
Supplies	9,708.03 / 175,893.54	Other	9,984.48 / 124,353.23

	This Qtr/Cumulative
Subtotal	68,762.87 / 822,216.79
Indirect Costs	36,413.81 / 435,692.25 (cumulative amount includes subaward indirects of $155,432.46)
Fee	_____/_____
Total	105,176.68 / 1,257,909.04

10. Comments on administrative and logistical matters.
A one year No Cost Extension was submitted 8/1/2013. The approval is still pending.

11. Use additional page(s), as necessary, to describe scientific progress for the quarter in terms of the tasks or objectives listed in the statement of work for this assistance agreement. See annual report

12. Use additional page(s) to present a brief statement of plans or milestones for the next quarter. See annual report